APPENDIX H-2

In Vivo Rodent Toxicity Reference Values Used to Assess the Accuracy of the 3T3 and NHK NRU Test Methods

Evaluation of the Candidate Reference Data

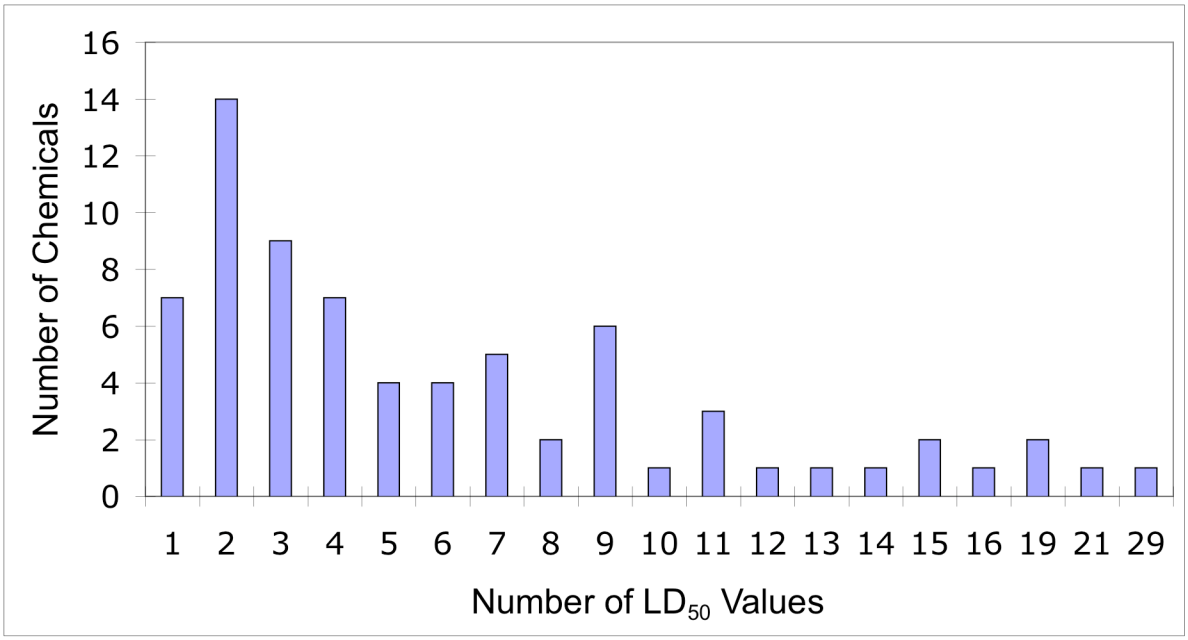
The 491 LD_{50} values identified by the literature search consisted of 485 rat oral LD_{50} values and six mouse oral LD_{50} values. Mouse oral LD_{50} values were used to determine reference values for colchicine, epinephrine bitartrate, and propylparaben since rat oral LD_{50} values for these three chemicals could not be located. Thirty rat oral LD_{50} values were believed to be duplicates of other reported values because the LD_{50} values and the experimental information matched exactly those cited by other publications from the same author(s) or because the same animal data were used to calculate multiple LD_{50} values (e.g., to evaluate various methods of calculation).

Two rat oral LD_{50} values provided by RTECS® were incorrect, possibly due to typographical errors. For the value of 200 mg/kg for acetylsalicylic acid, RTECS® cited a review by Diechmann (1969) that referred to a paper by Coldwell and Boyd (1966). Coldwell and Boyd (1966), however, actually reported an LD_{50} of 920 mg/kg. For sodium oxalate, RTECS® cited a review paper by Walum (1998) for an LD_{50} value of 11160 mg/kg. Although Walum (1998) provided no source, the LD_{50} is the same as that used in the MEIC study (Ekwall et al. 1998b). That LD_{50} was calculated from the LD_{50} for oxalic acid (Ekwall et al. 1998b), which is 7500 mg/kg according to RTECS®. The source for this figure, however, provides a value of 7.5 mL/kg of 5% oxalic acid (Vernot et al. 1977). Extrapolating this to sodium oxalate (MW = 134.0 g/mole vs 90.04 g/mole for oxalic acid) yields an LD_{50} of 558 mg/kg.

After exclusion of the 30 duplicate values and the two erroneous values for acetylsalicylic acid and sodium oxalate, 459 records remained for further evaluation. **Figure H2-1** shows the frequency of the number of LD_{50} values retrieved for the 72 chemicals. The number of LD_{50} values identified for any one chemical ranged from one to 29. The highest frequency was two LD_{50} values per chemical (14 chemicals). The highest number of LD_{50} values

retrieved for an individual chemical (acetonitrile) was 29. A large number of LD_{50} values were also identified for hexachlorophene (21), ethylene glycol (19), and carbon tetrachloride (19). Only one LD_{50} value was identified for seven chemicals: aminopterin, digoxin, epinephrine bitartrate, glutethimide, physostigmine, and propranolol HCl.

Figure H2 - 1 Distribution of the Number of LD_{50} Values Per Chemical



Protocols Used for the Candidate Reference Data

The LD_{50} data were collected using various protocols; however, information on the protocol details was often incomplete due to limited documentation in the reports. The 459 remaining data records exhibited the following characteristics:

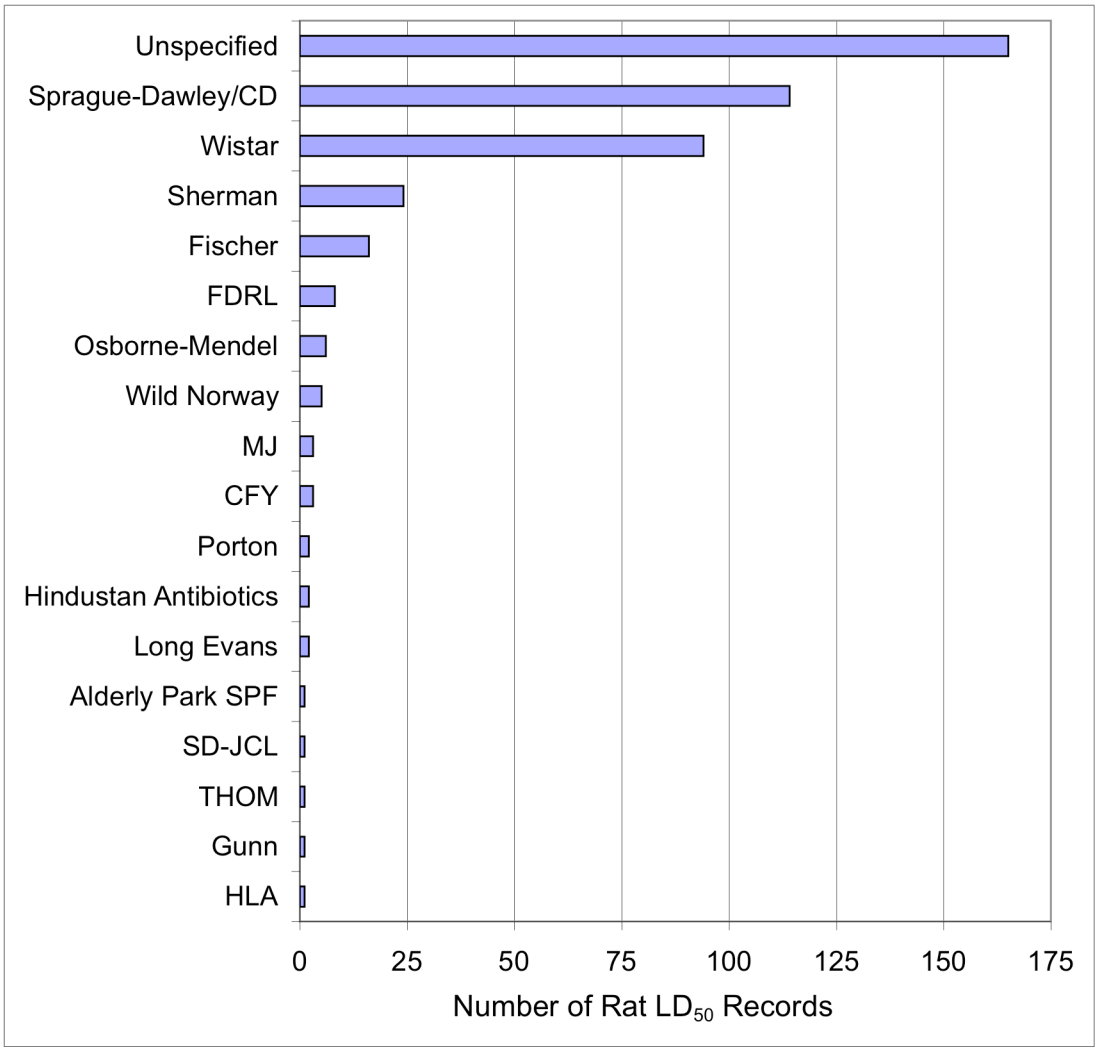
- 64% (293/459) specified the stock or strain of rodent used. The remaining 36% (167/459) that did not specify the stock/strain described rats as rats, albino rats, white rats, rats of different strains, and mice were described as mice.
- 63% (290/459) included age or weight information for the rodents.
- 77% (354/459) specified the gender of the rodent.
- 66% (305/459) stated the method used to calculate the LD_{50}.

- 48% (221/459) reported the number of rodents used at each dose and 47% (216/459) reported the total number of rodents used.
- 26% (118/459) specified the doses used.
- 14% (66/459) quantitatively specified the purity of the chemical used. Of the remaining records, 18% (83/459) described the purity qualitatively using such terms as "technical grade," "pure," "reagent grade," and "pharmaceutical grade," 11% (51/459) named only the source of the chemical, and 56% (259/459) provided no information on the chemical.
- 13% (61/459) reported the deaths at each dose.

Although many LD_{50} studies did not specify the strain or stock of rat used, the 293 studies that provided this information indicated that Sprague-Dawley/CD rats were the strain most frequently used (see **Figure H2-2**). Wistar rats were also frequently used. Strains such as Alderly Park, SD-JCL, THOM, Gunn, and HLA were the least frequently used. Of the six mouse LD_{50} values, the strain was unspecified for two studies. The other four LD_{50} values were obtained using CD-1, MS/Ae, dd, and B6D1F1(BDF1) mice.

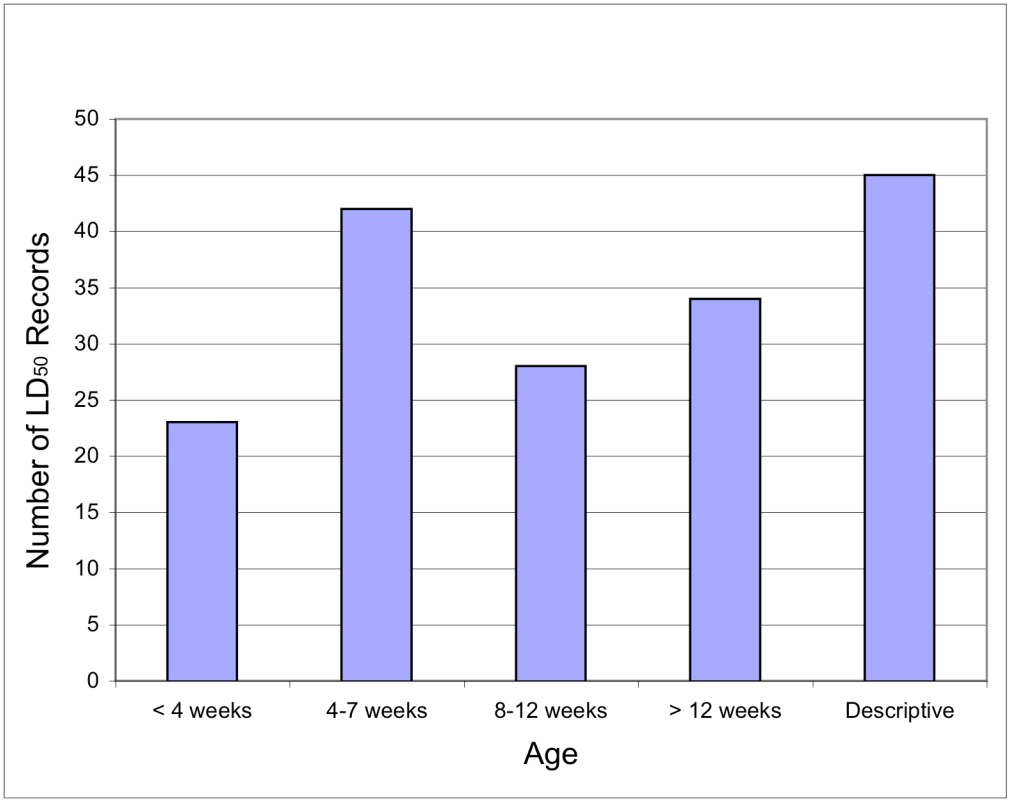
Of the 354 studies that reported rodent gender, the most frequently used gender for both rodents was male, which was used for 193 (55%) LD_{50} values. Female rodents were used for 104 (29%) LD_{50} values, both sexes were used for 55 (16%) LD_{50} values, and rodents of unspecified gender were used for 104 (29%) LD_{50} values.

Figure H2 - 2 Distribution of Rat Stocks/Strains



The age of the rodents used for the acute oral lethality studies also varied. Of the 174 LD_{50} studies that reported age, the most frequently used age was 4-7 weeks, which was reported for 42 (24%) LD_{50} values (see **Figure H2-3**). The majority of the reported ages were descriptive. Forty-five (26%) LD_{50} values used rodents that were described as young, adults, young adults, or older adults. Thirty (17%) LD_{50} studies used 8-12 week old rodents, which is the age recommended by current oral acute toxicity test guidelines (OECD 2001a, c, d; EPA 2002a). Twenty-three (13%) LD_{50} values were determined using rodents less than four weeks of age, and 34 (20%) LD_{50} values were determined using rodents greater than 12 weeks old.

Figure H2 - 3 Distribution of Rat and Mouse Ages



The duration of animal observation was not specified for 39% (179/459) of the LD_{50} reports. Of the 280 (61%) studies that reported the duration of observation, 136 (48%) reported an observation period of 14 days, which is recommended in the current oral acute toxicity test guidelines (OECD 2001a, c, d; EPA 2002a). The second most commonly used observation period was seven days, which was reported by 59 (21%) studies. Clinical signs were reported in 30% (137/459) of the studies.

Of the 305 studies that reported the method used to calculate the LD_{50} value, the most frequently used were the graphical log-probit methods such as Litchfield and Wilcoxon (1949), with 99 (33%) LD_{50} values, and Miller and Tainter (1944), with 24 (8%) LD_{50} values. The maximum likelihood probit method of Bliss (1938) and modifications were used for the calculation of 46 (15%) LD_{50} values. An additional 36 (12%) LD_{50} values were calculated using methods referred to in a general way as probit or log probit methods. The moving average method, such as that of Thompson (1947) or Weil (1952), was cited for 57

(19%) LD_{50} values. Thirteen (4%) LD_{50} values were described as being calculated by one method or another (e.g., by Weil or Litchfield and Wilcoxon), or by methods that were described generally, such as graphical or approximative. Some of the least frequently used methods were linear regression (six values), UDP (four values), and linear interpolation (one value). Estimates of variability such as confidence limits, standard error, or standard deviation were included in 62% (283/459) of the LD_{50} reports, but only 6% (28/459) included slopes.

Final Reference Values

Based on the study exclusion criteria described in **Section 4.1.2**, 73 (16%) of the 459 records identified were excluded. Thirty-one LD_{50}values were excluded because they were reported as ranges, 21 were excluded because the rats were less than four weeks old, five were excluded because the rats were feral, five were excluded because the rats were anesthetized, and four were excluded because the chemical administered was mixed with food. Additionally, four LD_{50} values for copper sulfate pentahydrate were excluded because very low purity (i.e., $\leq$ 20%) chemical was used. Three LD_{50} values were excluded because they were outliers at the 99% level (Dixon and Massey 1981) compared with the rest of the values for the particular chemical. These included one ethylene glycol value of 17,800 mg/kg (range of the other 16 values = 4000 - 9900 mg/kg), one meprobamate value of 794 mg/kg (range of other six values = 1286 - 1522 mg/kg), and one mercury chloride value of 160 mg/kg (range of other 10 values = 12 - 92 mg/kg). **Appendix H-1** provides the individual rationale for each LD_{50} value excluded by shading the cell that contains the reason for exclusion.

Triethylenemelamine, trichloroacetic acid, and xylene had the largest confidence limits in proportion to the geometric means. The confidence limits for triethylenemelamine and xylene were calculated from four LD_{50} values while those for trichloroacetic acid were calculated with five LD_{50} values. Nicotine and 2-propanol had the smallest confidence limits even though the number of values per chemical were similar to that for the chemicals with large confidence limits (nicotine N= 4, 2-propanol N = 6).